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Original article

Cell-mediated immunity in recent-onset type 1 diabetic children

Background: The ability to suppress an immune response makes regulatory T-cells (T-reg) an attractive candidate as a novel therapeutic agent for treating autoimmune diseases. The mechanisms involved in maintenance of peripheral tolerance include a specialized subset of regulatory-T-cells (T-reg) within the T-cell population. The $CD4^+$ $CD25^+$ T-cells may be important in modulating the risk for autoimmunity. Auto-reactive cytotoxic T-cells recognize peptide epitopes displayed on the beta cells surface in the context of HLA class I molecules. A population of $CD8^+$ regulatory T-cells characterized by expression of CD25 and FOXP3 have been identified and induced in the human peripheral blood cells. The regulatory activity of these cells is on autologous, antigen-reactive $CD4^+$ T-cells in a cell contact-dependent manner. These findings provide an evidence for a new mechanism for induction of immune regulation in human.

Objective: This study was aiming to assess the cellular immune parameters including the percentage of $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ ratio, $CD4^+CD25^+$, $CD8^+CD25^+$ lymphocytes, which may have its application in developing immune therapy based tools for halting disease progression.

Methods: This study was conducted on 20 children of recent onset type 1 diabetes (disease duration ≤ 6 months) who were compared to 10 healthy children. Each child was subjected to determination of CD markers by flow cytometer.

Results: The patients group shows a significantly lower $CD8^+$ lymphocyte percentage ($p < 0.01$) and significantly lower $CD8^+CD25^+$ lymphocytes percentage ($p < 0.05$) compared to control group. The $CD4^+$, $CD4^+/CD8^+$ ratio, $CD4^+CD25^+$ was not significantly different ($p > 0.05$) between the two groups. A significant inverse correlation was found between $CD4^+CD25^+$ T-cells and HbA1c percentage among patients group ($p < 0.05$). Also a significant difference in the percentage of $CD4^+CD25^+$ T-cells was found when patients with $HbA1c < 8\%$ were compared to those with $HbA1c \geq 8\%$ (the latter group had significantly lower percentage of $CD4^+CD8^+$ T-cells).

Conclusion: Type 1 diabetes is characterised at its onset by a lowered percentage of $CD8^+$ and $CD8^+CD25^+$ T-cells in peripheral blood, a normal percentage of $CD4^+$ and $CD4^+CD25^+$ T-cells. There may be an inverse correlation between percentage of $CD4^+CD25^+$ T-cells at disease onset and HbA1c level after three months. These data support the hypothesis that a defect in function or deficiency in number of T-regulatory cells may affect the pathogenesis of type 1 diabetes.

Keywords: Type 1 diabetes, cell-mediated immunity, children.

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) arises from selective progressive immunologically mediated destruction of the insulin-producing beta-cells in the pancreatic islets of Langerhans with consequent insulin deficiency.¹ Cell mediated immunity has been implicated in the pathogenesis of type 1 diabetes, auto-reactive T-cells that recognize islet auto-antigens have been identified and are thought to play a direct role in T1DM immuno-

pathogenesis.² A proposal supported by studies showing that administration of therapeutic agents that inhibit T-cell function delay disease progression in patients of type one diabetes. It is critical to note that auto-antibodies have no known etiologic role in diabetes and –simply put– are believed to represent the "smoke of the fire" in the pancreas and not the fire itself.³

Despite the understanding that type 1 diabetes pathogenesis is mediated by certain T-cells, detection of these specific T-lymphocyte subsets

remains largely elusive. Suitable T-cell assays are highly needed, since they could offer preclinical diagnosis and immune surrogate end points for clinical trials. Although CD4⁺ T-cell assays have met with limited success, CD8⁺ T-cells are increasingly recognised as key factors in the diabetes of the non-obese diabetic (NOD) mouse. CD8⁺ T-cells are likely to play a role also in humans and may provide new markers of beta-cell autoimmunity.⁴

T-cell tolerance is established centrally in the thymus and further strengthened and maintained through multiple mechanisms of peripheral tolerance.⁵ The manifest lack of self tolerance to beta-cell autoantigens in patients of type 1 diabetes compared with their non-diabetic counterparts therefore could be due, at least in part to a failure in one or more of these mechanisms. So recent interest has focused on a feature of tolerance that seems to bridge the central and peripheral processes, namely the CD4⁺, CD25⁺ regulatory T-cell.^{5,6} These cells represent a naturally occurring CD4⁺ T-cell population expressing CD25 that arises from the thymus and seeds into the periphery, creating a cohort of cells with profound T-cell immunosuppressive qualities. These cells can be detected in peripheral blood in humans and are able to suppress proliferation and cytokine production from both CD4⁺ and CD8⁺ T-cell in vitro in a cell-contact dependent manner. So a relative defect in terms of number or function, of these regulatory T-cells could contribute to the lack of self tolerance seen in patients of type 1 diabetes.^{1,7}

The initiation of T1DM appears to require cooperation between CD4⁺ and CD8⁺ T-cells, with CD4⁺ T-cells playing a key role in islet beta-cell destruction.⁸ Yet, neither in humans nor in NOD mice have the immunological requirements for diabetogenic CD8 T-cells been precisely defined.⁹ Pancreatic lymph node-derived CD4⁺ CD25⁺ T-reg cells were found to inhibit in situ differentiation of islet-reactive CD8⁺ T-cells into cytotoxic T-lymphocytes, thereby can help in preventing diabetes progression.¹⁰ The capacity of pancreatic lymph node (PLN) derived T-reg cells to regulate is dependent on TNF-related activation induced cytokine-receptor activator of NF Kappa B signals. Blocking of this pathway results in decreased frequency of CD4⁺ CD25⁺ T-reg cell in PLN, resulting in intra-islet differentiation of CD8⁺ T-cells into cytotoxic T-lymphocytes and rapid progression to diabetes.¹¹ CD8⁺ CD25⁺ T-reg cells are suppressor regulatory cells that require cell to cell direct contact for inhibition.¹² Hence, this study was designed to assess the cellular immune

parameters including the percentage of CD4⁺, CD8⁺, CD4⁺ CD25⁺ and CD8⁺ CD25⁺ T-lymphocytes in the peripheral blood in recent-onset type 1 diabetic children. This may possibly have its application in developing immune-therapy based tools for halting disease progression.

METHODS

Subjects: The present study was carried out at the Diabetes and Outpatient Clinics of Ain Shams University Children's Hospital, Cairo, Egypt, during the period from September 2006 till June 2007.

Patients' group: Comprised 20 patients with recent onset T1DM diagnosed within less than 6 months. They were randomly selected. They comprised 10 males and 10 females (ratio 1:1), with a mean age of 7.8 ± 3.5 years and a range of 4- 12 years. Patients were labelled to have T1DM on solid clinical grounds (ISPAD clinical practice guidelines, 2006/2007) i.e.:

- 1 Have age at onset of disease of 4-12 years.
- 2 Present with a dramatic classic triad of polyuria, polydipsia, and weight loss.
- 3 Present with ketoacidosis or ketonuria.
- 4 Require insulin to prevent metabolic deterioration with ketosis and ketoacidosis (i.e. are insulin dependent).

Control group: Comprised 10 healthy children. They served as controls. They comprised 6 males and 4 females at a ratio (3:2). They had a mean age of 5.5 ± 2.1 years with a range of 3-8 years.

Study measurements: The patients' group was subjected at diagnosis to detailed history taking, thorough clinical examination and laboratory investigations which included:

1. Complete blood picture by Beckman Coulter Gen. S system.
2. Blood chemistry including serum creatinine, urea and liver enzymes: ALT, AST by Synchron CX9 (Brea, California, USA) .
3. Lipid profile evaluation: serum total cholesterol (TC) and serum Triglycerides (TG) by Synchron CX9 (Brea, California, USA).
4. Assessment of HbA1c% after three months of start of treatment. It was assessed by high performance liquid chromatography technique.
5. Determination of the percentage of surface expression of CD4⁺, CD8⁺, CD4⁺ CD25⁺ and CD8⁺ CD25⁺ T-lymphocytes in the peripheral blood by flowcytometry, where two ml of venous blood were drawn from each patient and control subject into a tube containing K-EDTA as an anticoagulant. The monoclonal antibodies used included, CD4 fluorescein isothiocyanat, "FITC",

labelled, CD8 phycoerythrin, "PE", labelled, CD25 allophycocyanin "APC", labelled. Isotypic negative controls FITC, PE and APC, labelled were used to determine the non specific bridging. For each sample, one assay and one control tube were used. Fifty μL of whole blood were delivered in each tube, then five μL of monoclonal antibodies and isotypic controls were added in the test tube and the control tube respectively. The tubes were gently vortexed and incubated for 15 min at room temperature in the dark; cells were then washed in phosphate buffered saline (P.B.S). Stained samples were then treated with lysing solution (NH_4Cl buffered with KHCO_3 at pH 7.2), by adding 1.5 ml of it in each tube then incubated for 1-2 min at 37°C . The tubes were washed again prior to flow cytometric analysis. Analysis was done on a flow cytometer (coulter electronics Epics -XL-FI-USA) equipped with 480 nm air cooled Argon Laser. The percentage of single and double positivity stained cells were determined, data was plotted on four histograms. The first histogram was based on forward scatter versus side scatter where lymphocytes were gated. The second histogram measures the percentage of expression of CD4^+ , and CD8^+ (dual analysis). The third histogram measures the percentage of CD8^+ , CD25^+ percent expression. The fourth histogram measures the percentage of CD4^+ , CD25^+ percent expression.

Statistical Methods:

Analysis of data was done on an IBM computer system using SPSS (statistical program for social science) software. All numeric variables were expressed as mean \pm standard deviation (SD), Chi-square test was used to compare qualitative variables, Mann Whitney Willcoxon U test and Unpaired t-test were used to compare quantitative variables (the latter for independent variables) and Spearman correlation test was used to rank different variables against each other. A p-value of <0.05 was considered significant and p-value <0.01 was considered highly significant.

RESULTS

Both patients and control groups were comparable as regard age, sex and body mass index (BMI). The patients group had within normal mean of all studied parameters apart from high glycosylated hemoglobin (HbA1c), (8.3 ± 1.27), [Table 2]. Results of flow cytometry analysis were expressed as the percentage of CD4^+ T-cells, CD8^+ T-cells

and the percentage of the CD4^+ T-cells coexpressing CD25 and the CD8 T-cells which coexpress CD25. There were no significant differences in the percentage of CD4^+ lymphocyte or $\text{CD4}^+/\text{CD8}^+$ ratio in patients group (mean \pm SD, 33.4 ± 7.2 and 1.7 ± 0.8 , respectively) in comparison with control subjects (32.28 ± 12.2 and 1.2 ± 0.6), [Table 3].

Also, we found no statistically significant difference in the percentage of CD4 lymphocytes that coexpress CD25 in the patients group (2.9 ± 0.74) when compared with control group (2.77 ± 1.5). Furthermore, there was no significant difference between the two groups in the level of CD25 expression per cell, when expressed as the mean fluorescence intensity (MFI) of the CD4^+ CD25^+ cell population. The MFI of patients was (4.1 ± 0.9) and of control subjects was (3.7 ± 0.8).

A significantly low percentage of CD8^+ T-lymphocytes (22.1 ± 6.8) was found in the patients group as compared to the control group (30.6 ± 9.8), ($P < 0.01$). Moreover, there was statistically significant difference in the percentage of CD8^+ lymphocytes that express CD25 in patients of type 1 diabetes i.e. patients group (0.35 ± 0.31) as compared with control subjects (0.74 ± 0.87), [Table 3].

There was also statistically significant difference between the two groups in the level of CD25 expression per cell, when expressed as MFI of the CD8^+ CD25^+ cell population (38.6 ± 5.1) in patients with type 1 diabetes and (19.1 ± 2.1) in controls, [Table 3].

On correlating HbA1c values with the percentage of CD parameters, a significant inverse correlation was found between the level of HbA1c and the percentage of CD4^+ CD25^+ lymphocytes, but there were no significant correlations between the level of HbA1c and the percentage of CD4^+ , CD8^+ or CD8^+ CD25^+ lymphocytes [Table 4].

When patients with HbA1c value less than 8% and those with HbA1c value more than or equal to 8% were compared as regard the percentage of CD4^+ , CD8^+ , CD4^+ CD25^+ and CD8^+ CD25^+ lymphocytes, a significant high level of CD4^+ CD25^+ lymphocyte percentage was found in patients with HbA1c value less than 8% (i.e. of good glycemic control). However, there were no statistical significant difference between both groups ($<8\%$, $\geq 8\%$ HbA1c) as regard the other CD parameters in relation to glycemic control [Table 5].

Table 1. Comparison between patients and controls as regards clinical characteristics.

Variables	Patients N=20	Controls N=10	t/X ²	P
Age (yrs)	7.8 ± 3.5	5.5±2.1	t=1.9	>0.05
Gender				
Males	10 (50%)	6(60%)	X ² =0.3	>0.05
Females	10 (50%)	4(40%)		
Duration of illness (Weeks)	5.4 ± 6.9	-	-	-
BMI	19 ± 4.6	20.8±3.8	t=1.05	>0.05

BMI: Body mass index

P< 0.05: Significant test

Table 2. Description of different laboratory data of the studied patients.

Variables	Mean±SD
RBCs (Million/cmm)	4.14 ± 0.7
Hb gm/dl	11.9 ± 1.5
TLC x 10 ³ /cmm	7.7 ± 2.7
Platelets x 10 ³ /cmm	337.8 ± 98.9
Serum creatinine (mg/dl)	0.56 ± 0.26
ALT (IU/L)	22.9 ± 12.9
HbA1c (%)	8.3 ± 1.27
Serum cholesterol (mg/dl)	140.6 ± 46
TG (mg/dl)	99.2 ± 26

Table 3. The studied CD markers in patients and control groups.

Variables	Patients Mean±SD	Controls Mean ±SD	t	P
CD4+ (%)	33.4 ± 7.2	32.28 ± 12.2	0.31	>0.05
CD8+ (%)	22.1 ± 6.8	30.6 ± 9.8	2.8	<0.01**
CD4+CD25+%	2.9 ± 0.74	2.77 ± 1.5	0.24	>0.05
CD8+CD25+%	0.35 ± 0.31	0.74 ± 0.87	2.1	<0.05*
CD4+/CD8+%	1.7 ± 0.8	1.2 ± 0.6	1.3	>0.05
CD4 ⁺ CD25 ⁺ MFI	4.1 ± 0.9	3.7 ± 0.8	0.32	>0.05
CD8 ⁺ CD25 ⁺ MFI	38.6 ± 5.1	19.1 ± 2.1	2.3	<0.05

*: P< 0.05 = Significant test; **: P<0.01 = Highly significant test; MFI : mean fluorescence intensity

Table 4. The studied CD parameters and glycemic control among patients

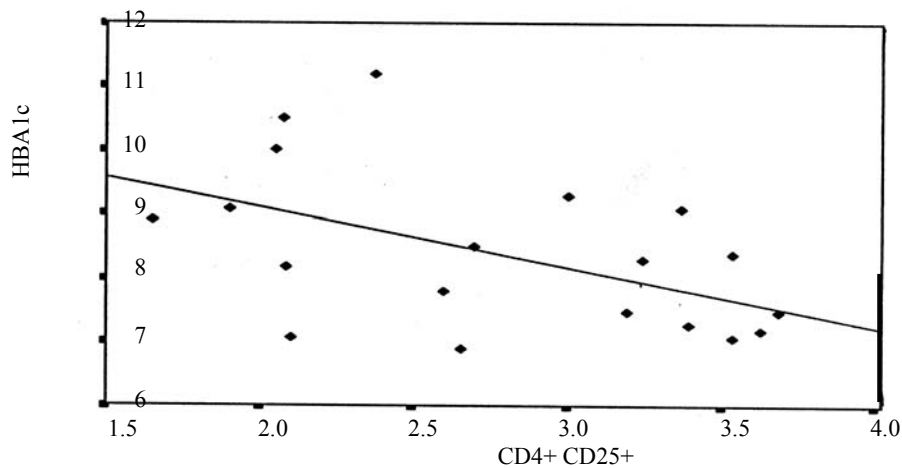
Variables	HbA1c %	
	r	P
CD4+%	-0.027	>0.05
CD8+%	0.08	>0.05
CD4+/CD25+%	-0.54	<0.05*
CD8+/CD25%	-0.14	>0.05

*: P< 0.05 = Significant test HbA1c: hemoglobin A1c

Table 5. Glycemic control and different CD markers.

Variables	HbA1c		P	Z
	≥8 %	<8%		
CD4+%	33 ± 8.7	33.8 ± 5.5	>0.05	0.24
CD8+%	22.8 ± 7.5	21.3 ± 6	>0.05	0.4
CD4+CD25+%	2.5 ± 0.6	3.2 ± 0.7	<0.05*	2.3
CD8+CD25+%	0.29 ± 0.2	0.42 ± 0.4	>0.05	0.9

*: P<0.05 = Significant test

**Figure 1.** Scatter diagram showing inverse correlation between HbA1c and the percentage of CD4⁺ CD25⁺ cells in the studied patients group (P<0.05).

DISCUSSION

In this study we aimed to assess percentage of cells thought to be involved in the autoimmune cell-mediated destruction of pancreatic β cells in the peripheral blood of recent onset type 1 diabetic children. We chose patients with recent onset type 1 diabetes, since most of them have ongoing insulinitis. As shown in table (3), we found no statistically significant difference in the percentage of CD4⁺ T-lymphocytes in the peripheral blood between the patient (33.4 ± 7.2) and control groups (32.28 ± 12.2 , $p>0.05$). This finding is in agreement with that of Milicevic et al.¹³, who studied β cell secretory function and percentage of different T-lymphocytes in the patients of early stage of type 1 diabetes mellitus (T1DM). They also found no statistically significant difference between patients (42 ± 9.4) and controls (44 ± 7.3) as regards the percentage of CD4⁺ cells ($p>0.05$). One possible explanation for this finding could be the shift of these cells from peripheral blood to pancreatic islet lymph nodes before disease onset to exert their action.¹⁴

In our study we also found that the percent of CD4⁺ CD25⁺ T reg cells did not show any significant difference between patients and controls

(2.9 ± 0.74 in the patient group versus 2.77 ± 1.5 in control group, $p>0.05$). There are several published reports analyzing peripheral blood CD4⁺ CD25⁺ T-reg frequency and function in patients with T1DM. Kukreja et al.¹⁵ found reduced T reg frequency, Lindley et al.¹⁶ and Brusko et al.¹⁷ found reduced T reg function, while Putnam et al.¹⁸ did not find any significant change as compared to healthy controls.. Our finding was in agreement with the results of other investigators^{16,19}, who found no significant abnormality in either the number of CD4⁺/CD25⁺ T-cell or the level of CD25 expression in patients of T1DM. This finding contrasts with an earlier published report suggesting that expression of CD25 is elevated in patients with T1DM,¹⁹ and another study demonstrating a decrease in CD25 expression on CD4T-cells.¹⁵ Also, our findings were in agree with observations published by Gessl and Waldhauser.²⁰

Factors that could account for these differences among studies include the definition of CD25⁺ cells, disease duration, and most importantly, appropriate matching for age and HLA-type in control population. As CD25 is expressed by both activated and regulatory T-cells, so careful interpretation of results is required. By its nature, a

cell type that negatively regulates other immune effectors is difficult to identify with precision at the single cell level. For this reason, the identification of a phenotype, CD25 positivity which defines a population of cells with regulatory properties both in vivo and in vitro, has been heralded as an important advance.^{6,21}

CD4⁺ CD25⁺ T-reg cells are suppressor cells (suppress CD8⁺ cytotoxic T cells) so they are actually expected to decrease in T1DM. Significant inclusion of other cells such as recently activated CD4⁺ pathogenic T-cells in the analyzed sample as they also fall in this phenotypic subset (T-reg cells) may alter the results. Only T-reg cells express the transcription factor fork head box protein 3 (Foxp3). Recent studies suggest that almost half of the bulk of CD4⁺CD25⁺ T cells detected in humans do not express Foxp3 (the gene responsible for differentiation of T-reg cells) when the gate is set to include the top 10–15% of the CD25⁺ cells. Even when the top 5% of the CD25⁺ cells are gated, significant contamination with Foxp3– cells is still present in some samples. The top 2% CD25 bright gate most reliably identifies a highly enriched Foxp3+ population, but significantly underestimates the frequency of Foxp3+ cells in most individuals.¹² Therefore, the use of more markers such as Foxp3 may increase the specificity of detection of only CD4⁺CD25⁺ T-reg cells and alter results.

Our findings corroborate with similar studies in other autoimmune conditions such as multiple sclerosis and autoimmune polyglandular syndrome type II, which also report a defect in CD4⁺/CD25⁺ cell function and not the number of this type of cells.^{22,23} Taken together these studies may point to a central role for this regulatory cell population in the development of a wide range of autoimmune conditions.

A significantly lower percentage of CD8⁺ T-lymphocytes as compared to controls (22.1 ± 6.8 versus 30.6 ± 9.8) $p < 0.01$ was found. This finding was in agreement with Avanzini et al.¹⁴, who studied the percentage of the CD4⁺ and CD8⁺ T-cells in T1DM at disease onset and after 15 months and eight years. They too found lower percentage of peripheral CD8⁺ cells at onset of disease as compared to controls. They suggested that this may reflect the initial decrease in cytotoxic T cells in the blood which occurs as these cells migrate to the pancreatic islets where the destruction is occurring (starting from the phase of insulinitis before disease onset). In fact Wong et al.²⁴, studied the structural and functional relationships of autoantigenic peptides of insulin that stimulate CD8⁺ T-cells in T1DM. They showed that CD8⁺ T-cells recognizing

insulin B15-23 peptide are present in the very early phases of insulinitis. CD8⁺ T-cells were seen to be predominant in islets and low in peripheral blood in patients with newly diagnosed diabetes who underwent pancreatic biopsy.²⁵

It has reported that untreated acute diabetes causes rapid lymphopenia followed by homeostatic T-cell proliferation. This diabetes induced lymphopenia was associated with an immuno-suppressed state that could be sufficiently strong to allow engraftment of fully allogenic beta cells or block rejection of islet transplants.²⁶

The finding of significantly lower percentage of CD8⁺ T-lymphocytes as compared to controls in this study came in disagreement with the work of Milicevic et al.¹³, found no difference between diabetics and controls as regards percentage of the CD8⁺ T-cells. We can attribute the difference between our study and theirs to the difference in timing of the study in relation to the time of diabetes diagnosis. Most blood samples in our patients were taken within days to weeks of initial presentation with diabetic ketoacidosis (DKA) at disease onset (mean disease duration in our study 5.4 ± 6.9 weeks) while in Milicevic et al.¹³ study, they were taken over a period of several months (6 months) respectively.

Also, we found that the percentage of CD8⁺CD25⁺ T-lymphocytes was significantly lower in the peripheral blood among patients as compared to controls ($0.35 \pm 0.31\%$ versus $0.74 \pm 0.87\%$, respectively, $p < 0.05$). These cells are suppressor cells that act by direct cell to cell contact in the pancreatic islets so they leave the peripheral blood to pancreatic islets to exert their effect.¹² Bisikirska et al.²⁷ studied T-cell receptor (TCR) stimulation with modified anti-CD3 monoclonal antibodies in patients with T1DM. They too, documented in their study a low percentage of CD8⁺CD25⁺ T-cells in patients with recent onset T1DM and an increase in this percentage after stimulation with anti CD3 monoclonal antibodies. This decrease in CD8⁺ CD25⁺ may be due to firstly the fact, that although both CD4⁺ CD25⁺ and CD8⁺ CD25⁺ are regulatory cells CD8⁺ CD25⁺ T-reg cells act by direct cell to cell contact so their action is exerted within the lymph nodes at the islets of pancreas.¹² Secondly, the CD8⁺ markers is expressed only on the surface of natural killer cells in addition to cytotoxic T-cells as compared to CD4⁺ which is expressed on many cells.²⁸ Moreover, the number of CD4⁺ T-cells normally is double that of CD8⁺ T-cells, so any decrease in the percentage of CD8⁺ CD25⁺ T-cells will have more reflection on the assayed percentage than that of CD4⁺ CD25⁺ T-cells. In fact in our

study CD4⁺/CD8⁺ ratio showed a mild, though non-significant increase.

As evident from table (4), a positive inverse correlation was found between the degree of glycemic control (as reflected by decreased levels of HbA1c) and the percentage of CD4⁺ CD25⁺ T-lymphocytes but not with the percentage of other CD parameters (CD4⁺, CD25⁺ and CD25⁺) among the studied the patient. This means that the higher the level of CD4⁺ CD25⁺ T-cells the patient had, the lower was his HbA1c% after 3 months and vice versa. Furthermore, HbA1c value below 8% was associated with a higher percentage of CD4⁺, CD25⁺ T-cells when compared with HbA1c value of more than or equal to 8% and a significant statistical difference was found as regards the HbA1c value and percentage of CD4⁺, CD25⁺ T-cells, ($P < 0.05$). This may be due to the fact that CD4⁺ CD25⁺ T-cells are regulatory cells which can inhibit in situ differentiation of islet-reactive CD8⁺ T-cells into cytotoxic T-lymphocytes (that are responsible for beta cells destruction), so the higher the percentage of CD4⁺ CD25⁺ T-cells, the more the preserved well functioning beta cells, the better the control of the disease, and consequently the better the glycemic control as compared to those with a lower percentage of CD4⁺ CD25⁺ T-reg cells.¹⁰ Another study¹⁴, found no correlation between the percentage of various cells and the HbA1c level. Yet, in their study the HbA1c together with the cell quantitation were done together at diagnosis, then after 15 months and after 8 years of disease onset. In our study cell assay was done at disease onset and the HbA1c assessment was carried after 3 months to have a better idea about disease control. So, in interpreting and comparing results one should keep in mind the fact that the percentage of various cells changes with time.²⁹

To conclude T1DM as an autoimmune disease, is characterized at its onset by a lower percentage of CD8⁺ T-cells, a lower CD8⁺ CD25⁺ in the peripheral blood, and a normal percentage of CD4⁺ T-cells. There is a close inverse correlation between the percentage of CD4⁺ CD25⁺ T-cells at disease onset and the HbA1c value after 3 months. Suggesting a beneficial role for the early use of immunomodulation in halting the progression of the disease. Evaluation of the various T-cell subsets (CD4⁺, CD8⁺, CD4⁺ CD25⁺ and CD8⁺ CD25⁺ T-cells) in patients at risk for developing of type 1 diabetes together with using of more specific monoclonal antibodies as FOXP3 for detection of CD25⁺ T-cells seems worthwhile. It would give a better insight on the future development of diabetes. This, together with the use of specific monoclonal

antibodies against cells involved in the inflammatory destruction of islet cells deserve further studies.

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